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ASSOCIATION EURATOM - T.N.O.

(European Atomic Energy Community and Stichting voor
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PATHOLOGY OF SECONDARY DISEASE IN PRIMATES

by

M.J. de VRIES

1964

Work performed under the Euratom contract No. 004-59-12 BIAN

**Reprinted from the
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PROCEEDINGS OF THE
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THERAPY AND CHEMICAL PROTECTION
IN IRRADIATED PRIMATES 1962

PATHOLOGY OF SECONDARY DISEASE IN PRIMATES

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Secondary disease of radiation chimeras was extensively studied primarily in mice. The etiology of the disease was analysed by various experimental approaches and found to be related to an immunological reaction of grafted immunologically competent cells against the host (the evidence has been reviewed several years ago by van Bekkum et al. (1959). Although a variety of characteristic lesions have been described, death in these mice appears to be almost uniformly due to infectious disease (van Bekkum et al., 1959; de Vries and Vos, 1959). The susceptibility to infections seems to be indirectly related to the graft anti-host reaction, since it is most readily explained by the generalized atrophy of the lymphatic tissues in these animals. As will be discussed later, this lymphatic atrophy may be considered to be a direct consequence of the interaction between reacting donor cells and host antigen.

Other characteristics of secondary disease in such bone marrow treated mice are its late appearance and chronic course and the fact that, depending on host-donor combination, a certain proportion of these mice may ultimately survive in the presence of permanent chimerism (an extensive discussion of the changes in these old chimeras and the development of immunological tolerance of the graft towards the host in these mice will be published else-

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where by van Bekkum et al., 1962). Lastly, an important feature is, that the survival may be greatly enhanced by treatment with antibiotics (van Bekkum and Vos, 1961).

In contrast to the results obtained with mice, it was found from limited experience in man and more extensive experience in monkeys, that grafting of homologous bone marrow in irradiated primates is uniformly lethal if permanent chimerism is successfully obtained (Mathé et al., 1960; Crouch et al., 1961). The existence of chimerism was determined by serological typing of erythrocytes or sex chromatin counts of neutrophils. This lethality may also be attributed to secondary disease mainly because of the following two observations. Treatment with autologous or monozygotic twin bone marrow can be successful; secondly, the lesions seen after homologous marrow transplantation in particular those of the skin and the liver parenchyma, are highly similar to those found in mice with secondary disease (de Vries et al., 1961).

In primates, however, the disease has a much more acute course and is apparantly of a very severe character. Moreover, infectious disease cannot explain death in a substantial proportion of cases and antibiotics are not effective in preventing death (Crouch et al., 1961).

It has been put forward, that the pattern of secondary disease in bone marrow treated primates is highly reminiscent of the acute mortality obtained in irradiated mice treated with lymphoid cell suspensions in addition to bone marrow (de Vries et al., 1961). The similarity was stressed when a number of lesions present in primate chimeras were seemingly duplicated in these mice (de Vries, to be published). These observations led to the provisional conclusion that mortality caused by secondary disease in primates is pathogenetically more directly related to graft anti-host activity than in bone marrow treated mice and secondly, that lymphoid cells are of prime importance in its production. In the following paragraphs morphologic-

al evidence in favour of these assumptions will be presented.

The most important lesions found in primate chimeras are briefly reviewed in table I. These lesions are accompanied by bone marrow regeneration (plate 1), which clearly distinguishes the syndrome from graft rejection.

Table I

PATHOLOGY OF SECONDARY DISEASE

Bone marrow regeneration.
Regeneration, necrosis and atrophy of lymphatic tissues.
Dermatosis: acanthosis, follicular hyperkeratosis, parakeratosis, dyskeratosis, vacuolar degeneration, liquefaction degeneration of basal layer, metaplasia of excretory ducts of sweet glands, atrophy.
Loss of crypt and surface epithelium in the intestines.
Liver necrosis and isolated cellular degeneration in other epithelial tissues.
Infectious disease.

A dermatosis is found, characterized by either acanthosis (plate 2 and 3) or atrophy of epidermis and hair follicles (plate 4). A number of degenerative changes of the epithelium are also apparent: premature keratinization of cells (dyskeratosis, plate 5, 6 and 17), liquefaction degeneration of basal cells (plate 6) and vacuolar degeneration in the malpighian layer, sometimes leading to reticular degeneration and the formation of multilocular vesicles (plate 4). Finally, follicular hyperkeratosis and parakeratosis are characteristic (plate 2 - 4).

In almost all cases examined, severe intestinal changes are present. Extensive disintegration of cells in the glandular crypts of the intestinal mu-

cosa occurs (plate 7), leading to the loss of all epithelial elements, i. e. intestinal denudation (plate 8 and 9). Although the colon and ileum are most severely affected, lesions may be present in the entire intestinal tract, including the stomach (plate 10). The intestinal lesions to a certain extent do resemble those known to be caused by radiation alone, but several arguments may be put forward, which plead strongly against such an interpretation (de Vries et al., 1961). The most important of these are that the extent of the lesions is out of proportion with respect to the radiation dose, the lesions occur later than would be expected if the radiation were responsible and they are not found in irradiated non-treated monkeys neither in monkeys treated with autologous bone marrow after similar radiation doses (table II).

Table 2

PATHOLOGY OF IRRADIATED MONKEYS TREATED WITH BONE MARROW

	Irradiated non-treated	Autologous bone marrow	Homologous bone marrow		
			T*	ID*	NT*
Bone marrow regeneration	0/7	8/11	11/12	2/2	0/3
Hemorrhagic necrosis colon	6/7	0/11	0/12	0/2	2/3
Septichemia	4/7	0/11	0/12	0/2	0/3
Other infecteous diseases	1/7	3/11	8/12	1/2	0/3
Denudation intestines	0/7	1/11**	10/12	0/2	0/3
Massive liver necrosis	0/7	0/11	3/12	0/2	0/3
Jaundice	0/7	0/11	5/12	2/2	0/3
Dermatitis	0/7	0/11	8/10	-	-
Regeneration lymphatic tissues	1/7	7/11	6/12	0/2	0/3
Necrosis lymphatic tissues	0/7	0/11	3/12	0/2	0/3

* T = Take
ID = Insufficient data as determined by sex chromatin counts of neutrophils
NT = No take

** Monkey irradiated with 1065 r

Dissociation and necrosis of liver parenchyma and a variety of bacterial, mycotic, helminthic and possibly also viral infections have been found in a number of chimeras.

Other lesions than the above mentioned may be found in secondary disease, but will not be further digressed upon at this moment. For the present discussion it is only of importance to summarize the essential features which all lesions have in common.

1. Degeneration of cells, mainly in epithelial tissues, as already indicated in the previous paragraph. This may be massive in the intestines, the skin and the liver. Diffuse isolated degeneration of cells has been found, however, in a great number of other sites: the epithelium of the oesophagus (plate 12), renal pelvis, salivary glands, exocrine and endocrine parts of the pancreas, adrenals, Fallopian tube (plate 13) and bile ducts (plate 14 and 15).

   Cell death is indicated by a number of changes: karyorrhexxis (plate 7, 3, 14 and 15) and pyknosis of nuclei (plate 7), vacuolar degeneration (plate 4, 6, 12 and 17) and increased eosinophilia of the cytoplasm (plate 14). In the skin premature keratinization of epidermal cells has been mentioned (plate 5, 6 and 17) which may also be interpreted as a mode of cell death. Atrophic changes may supervene in the most severely affected tissues (plate 4 and 7).

2. Concurrently with the regressive changes, regeneration usually occurs, which, depending on the site, may or may not be able to restore the integrety of the tissues affected. The regeneration is indicated by an increase of mitotic frequency, which is most conspicious in tissues in which normally mitoses are not easily found (plate 13). The regenerative activity often gives rise to a hyperplastic appearance of glands or other epithelial tissues (plate 2, 3, 6, 7 and 10).

3. The affected tissues generally are more or less heavily infiltrated with lymphoid cells (plate 4, 6, 7, 10, 12, 15 and 16). Sometimes small numbers of these cells are seen to have penetrated into the epithelium while the epithelial cells adjacent to the invading cells display the various degenerative changes described before (plate 6, 7, 10, 12 and 17). In addition one often gets the impression that the intra-epithelial lymphoid cells also disintegrate, although this is difficult to verify.

4. A most significant sequence of changes is seen in the lymphatic tissues proper, i.e. the cortex of the lymph nodes and the splenic follicles. Following the radiation atrophy (plate 18), extensive regeneration occurs in a number of animals, mainly at the end of the first week (plate 19 and 20). Many mitotic figures are noted and in addition large numbers of reticular cells, stem cells and immature lymphoid cells distend the available tissue space.

   At the end of the first week and in the second week the regenerated lymphatic tissues of a number of monkeys examined in this period, show massive disintegration of lymphoid cells (plate 21). The characteristic feature is, that the necrosis does not involve the stromal supporting tissues and the vascular endothelium.

   The lymphatic tissues of chimeras which survived beyond the first 2 weeks are almost universally severely depleted of lymphoid cells. The atrophic lymph nodes and splenic follicles are made up of an empty reticular stroma and distended sinusoids, in which only few lymphocytes and histiocytic cells and a variable number of plasma cells are distributed (plate 22).

Before we attempt to interprete the given set of morphological changes, it is necessary to review a few relevant facts concerning the function of lymphoid cells and their fate in chimeras.

Pertinent to the observation of lymphoid cell infiltration in the diseased tissues of primate chimeras, is the evidence that a skin homograft is rejected by a cell mediated immunological reaction (Brent, 1958). The immunologically active cell is believed to be the small lymphocyte (Gowans et al., 1961). Of similar significance is the finding, that the pathological changes in homologous skin grafts, which are being rejected, resemble in many details those occurring in the skin of radiation chimeras, as described earlier in this paper (de Vries, to be published). The assumption seems to be justified, that in the primate radiation chimera, certain host tissues are being "rejected" in a somewhat similar way by the homologous transplant of lymphoid cells. With the necessary reservations these assumptions are supported by the following observations in rodent chimeras. The lymphatic tissues of mice treated with rat bone marrow appear to be repopulated by lymphoid donor cells (Brocades Zaalberg and van Bekkum, 1959). Moreover, autoradiography has shown, that when donor lymphoid cells labeled with tritiated thymidine are injected into new-born rats (Porter and Cooper, 1962) or into 5-week old F_1 hybrid rats, respectively, (Gowans et al., 1961) labeled cells are found to migrate not only into the lymphatic tissues proper, but also into the intestinal mucosa. Experiments in which similarly labeled cells were used have suggested that the same occurs in irradiated F_1 mice treated with parental spleen cells (Balner, 1962). In this context it should be recalled that the mucosal epithelium belongs to the most severely affected tissues in primate chimeras.

Gorer and Boyse (1959) have described a destruction of the lymphoid tissues of the host in (non-irradiated) (C57BL x A)F_1 mice treated with isoimmune parental strain cells. They postulated that the rapid disappearance of C57BL lymphoid cells, as noted in their experiments, is due to damage by the excess of antigen in the host environment to which these cells are exposed. This would indicate that, in the graft anti-host reaction not only the target

cells but also the antibody-forming cells are being destroyed.

With these data in mind, we may now attempt a tentative reconstruction of the set of events leading to secondary disease and death in primate radiation chimeras.

In the available space in the lymphatic tissues, caused by the radiation induced cellular depletion, a repopulation by lymphoid donor cells, derived from the injected bone marrow, occurs. In the absence of sensitization the proliferation at first takes place unimpeded and is possibly even promoted by antigenic stimulation, leading to the formation of a pool of potentially immunologically competent cells. Subsequently cells from these lymphatic organs migrate to peripheral tissues.

During the second part of the first week sensitization occurs. The sensitized cells penetrate the epithelium of a variety of organs and tissues and severely damage their epithelial cells, either by the secretion of anti-body or by non-specific cytotoxic products liberated during the desintegration of donor lymphoid cells after interaction with host cells (so-called enzymatic destruction as suggested by Amos, 1960). Similarly because of the interaction between host antigen and lymphoid donor cells massive destruction of the latter ultimately occurs in the lymphatic tissues.

The loss of epithelial cells induces a process of repair, which seems to be at least partially successful in some tissues, but apparently cannot compete with cell destruction in others such as the intestinal crypts, where extensive denudation of the mucosa occurs.

The death of the host may be explained by the consequences of intestinal denudation and possibly by toxic factors due to the wide-spread destruction of both host and donor cells.

One of the facts that still demand an explanation is, why the intestinal crypt cells are so particularly sensitive to the graft anti-host reaction. One factor could be the combination of radiation induced and immunological

damage, as has been discussed in a previous paper (de Vries et al., 1961). Another may be the circumstance that the intestinal tract is in fact a lymphatic organ, and moreover, a lymphatic organ in which epithelial tissue is intimately associated with lymphoid cells. In the chimeric intestine, the epithelial target cells are obviously surrounded by the pool of immunologically active donor cells.

If our assumptions are correct the conclusion must be drawn that the whole problem of the early death of the primate chimera centres around the lymphoid donor cells. In primates treatment with homologous bone marrow results in an early unrestrained proliferation and accumulation of donor lymphoid cells, presumably accounting for the inevitable early lethality. In those homologous host-donor combinations in mice treated with marrow only, in which severe secondary disease occurs, this early repopulation is not apparent to the extent that it is seen in primates. As will be recalled secondary disease in these mice develops in a much later phase. Evidently not the primary effects of the graft anti-host reaction, but the secondary atrophy of the lymphatic tissues endangers the life of these mice by the increased risk of infection. It seems therefore, that this late form of secondary disease may be self-limiting, due to the fact that lymphoid cells newly produced by the graft, which has been sensitized by that time, are continuously eliminated due to the excess of host antigens.

As will be discussed in the next paper, one solution of the dilemma would be, to search for methods to eliminate selectively lymphoid cells from bone marrow suspensions of primates. It might be expected that monkeys treated with such suspensions would still develop the late form of secondary disease, as it occurs in bone marrow treated mice. One could hope, however, that primates treated in that way, survive the early dangerous phase and be effectively treated during the late phase of secondary disease which might ensue.

Another more attractive approach would be an in vitro induction of immunological tolerance of donor lymphoid cells towards the prospective host. Results of experiments by van Putten with mice (van Putten, 1962) give rise to the hope that large amounts of tolerant lymphoid cells may successfully compete with similar non-tolerant cells present in primate bone marrow suspension.

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REFERENCES

Amos, D. B. Ann. N. Y. Acad. Sci., 87 (1), 273, 1960.

Balner, H. Unpublished observations, 1962.

Bekkum, D. W. van, Vos, O., and Weyzen, W. W. H. J. Natl. Cancer Inst. 23, 75, 1959.

Bekkum, D. W. van, and Vos, O. Int. J. Rad. Biol., 3, 173, 1961.

Bekkum, D. W. van, Putten, L. M. van, and Vries, M. J. de. Ann. N. Y. Acad. Sci. 1962 (in press).

Brent, L. Progress. Allergy, 5, 271, 1958.

Brocades Zaalberg, O., and Bekkum, D. W. van. Transpl. Bull. 6, 91, 1959.

Crouch, B. G., Putten, L. M. van, Bekkum, D. W. van, and Vries, M. J. de. J. Natl. Cancer Inst., 27, 53, 1961.

Gorer, P. A., and Boyse, E. A. Immunology, II no. 2, 182, 1959.

Gowans, J. L., Gesner, B. L., and McGregor, D. D. In CIBA Foundation Study Group no. 10 on "Biological activity of the Leucocyte" (G. E. W. Wolstenholme and M. O'Connor, editors) London, J. and A. Churchill, Ltd. 1961, pp. 32.

Mathé, G., Bernard, J., Vries, M. J. de, Schwarzenberg, L., Larrieu, M. J., Lalanne, C. M., Dutreix, A., Amiel, J. L., and Surmont, J. Revue Hématol., 15, 115, 1960.

Porter, K. A., and Cooper, E. H. J. Exptl. Med. 115, 997, 1962.

Putten, L. M. van. Transpl. Bull., 29, 421, 1962.

Vries, M. J. de, and Vos, O. J. Natl. Cancer Inst., 23, 1403, 1959.

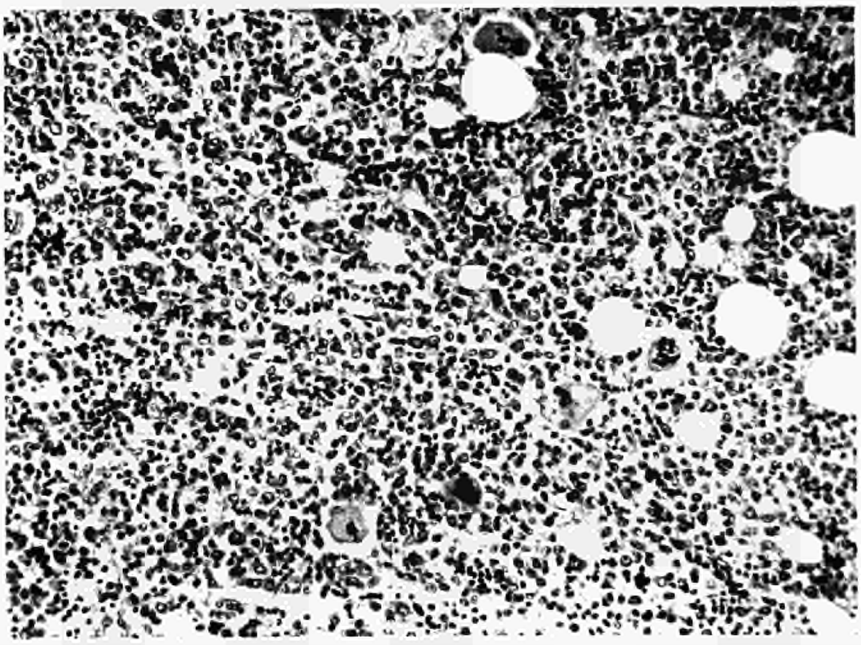
Vries, M. J. de, Crouch, B. G., Putten, L. M. van, and Bekkum, D. W. van. J. Natl. Cancer Inst., 27, 67, 1961.

Vries, M. J. de. Transpl. Bull., 30, 125/13, 1962.

Vries, M. J. de. To be published.

Plate 1 Bone marrow regeneration in monkey, 26 days after irradiation (700 r) and treatment with homologous bone marrow (11.2×10^8 cells).
Hematoxylin and eosin x 190

Plate 2 Dermatosis in same monkey as in plate 1. Note acanthosis, severe follicular hyperkeratosis and lymphoid cell infiltration of dermis in general and surrounding the hair follicles.
Hematoxylin and eosin x 30



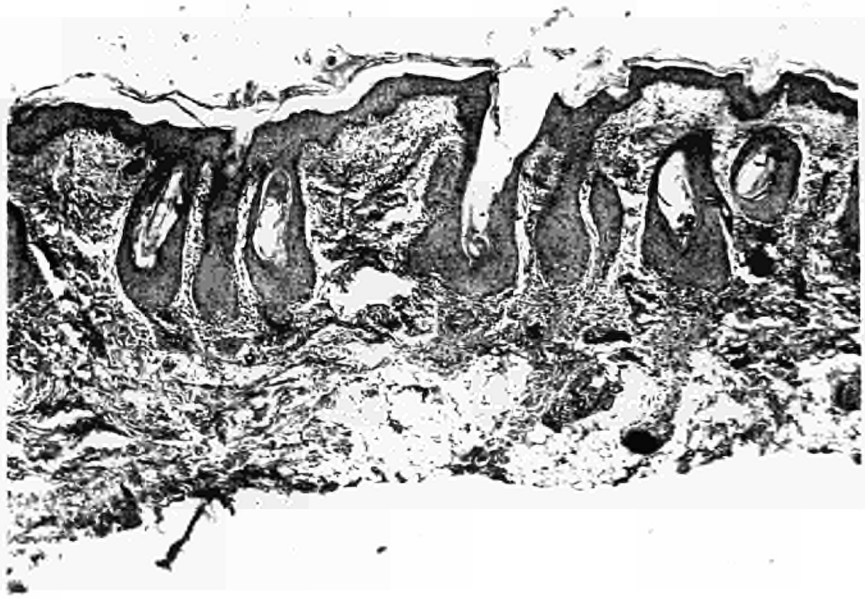
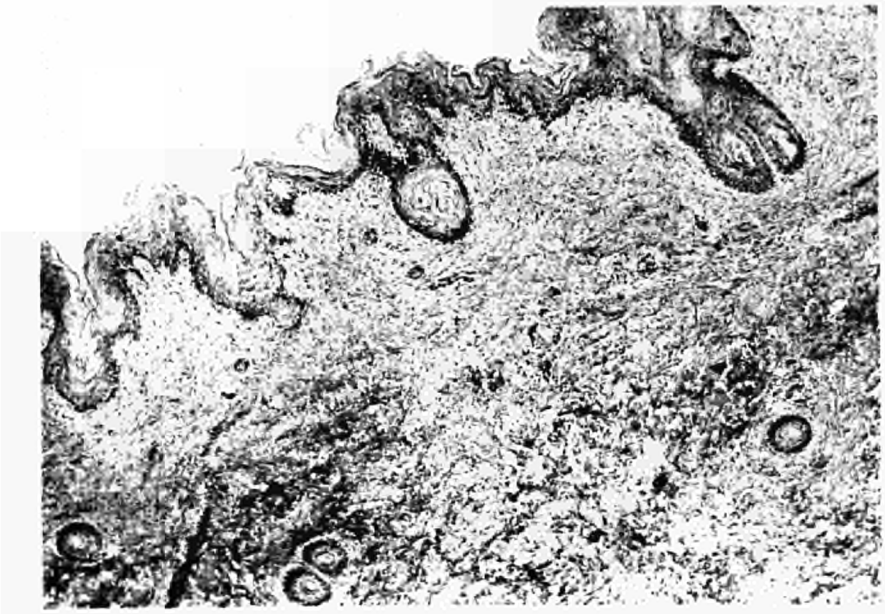


Plate 3 Dermatosis in a leukemic child, 29 days after 880 r Co^{60} γ-irradiation and 19 days after treatment with 11.5 x 10^9 bone marrow cells from its mother. Acanthosis and follicular hyperkeratosis.
Hematoxylin and eosin x 40
(courtesy of G. Mathé)

Plate 4 Dermatosis in monkey, 19 days after irradiation (600 r) and treatment with homologous bone marrow (15.2 x 10^8 cells). Parakeratosis. An atrophic hair follicle is present to the right. Vacuolar degeneration in stratum malpighii, leading to formation of clefts and small vesicles. Lymphoid cell infiltration of dermis, several of these cells have penetrated the epithelium.
Hematoxylin and eosin 190 x



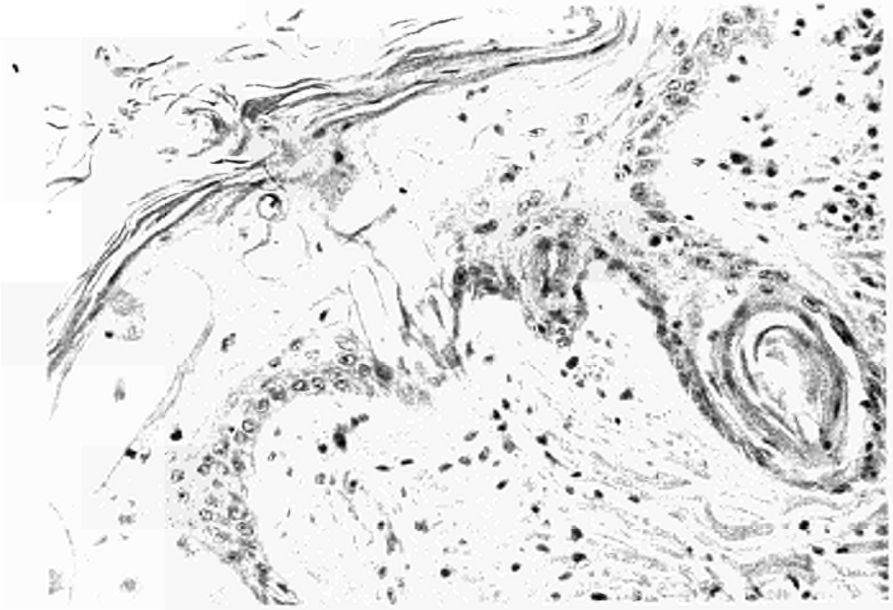


Plate 5 Dermatosis in same child as in plate 3. In the epidermis 3 dyskeratotic cells are present.
Hematoxylin and eosin x 300

Plate 6 Dermatosis in same child as in plate 3. Lymphoid cell infiltration of dermis accompanied by extensive liquefaction degeneration of basal layer. Acanthosis. A number of dyskeratotic cells may also be seen.
Hematoxylin and eosin x 190

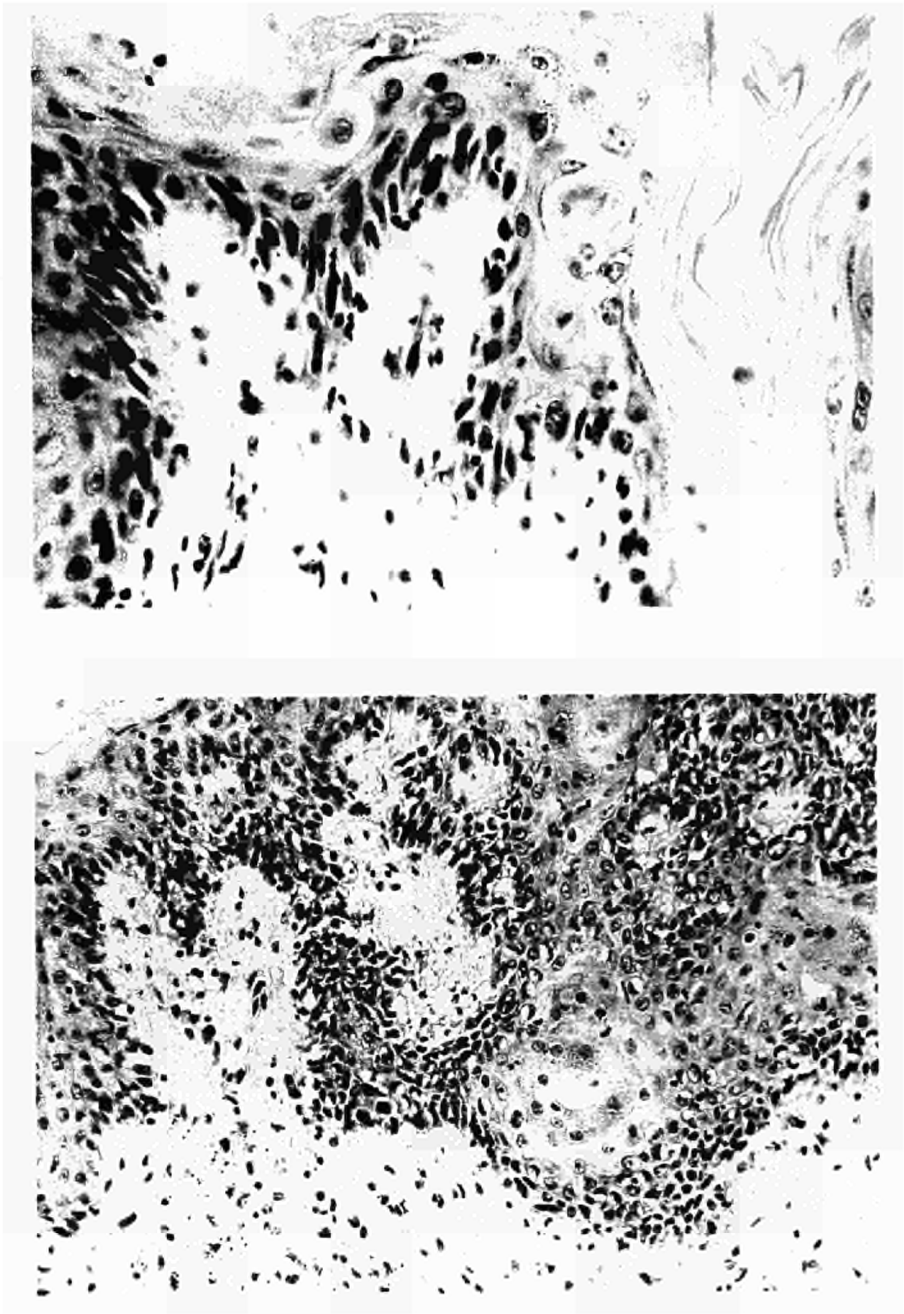
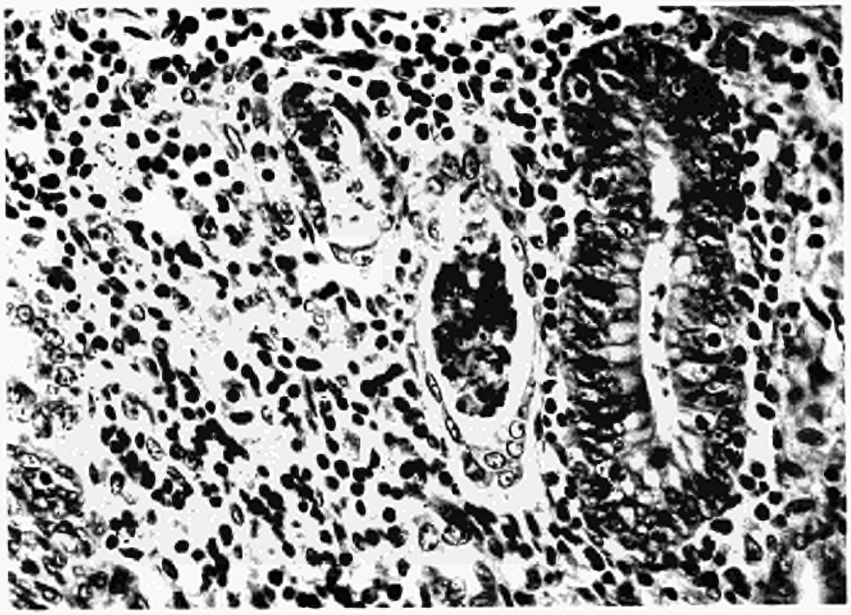


Plate 7 Wide-spread disintegration of crypts in the colon of monkey 7 days following irradiation (700 r) and treatment with homologous bone marrow (28 x 10^8 cells). Note heavy lymphoid cell infiltration of lamina propria and invasion by several of these cells of the epithelium of a hyperplastic crypt to the right. In this crypt several mitoses as well as diffuse pyknosis and karyorrhexxis of epithelial cell nuclei may be seen. Two other crypts are atrophic and show accumulation of degenerated and desquamated cells in the lumen.

Hematoxylin and eosin x 300

Plate 8 Complete loss of crypt and surface epithelium in the ileum of monkey 7 days after irradiation (750 r) and 6 days after treatment with homologous bone marrow (35 x 10^8 cells) which had been stored in an ice-box at 4° C for 48 hours.

Hematoxylin and eosin x 30



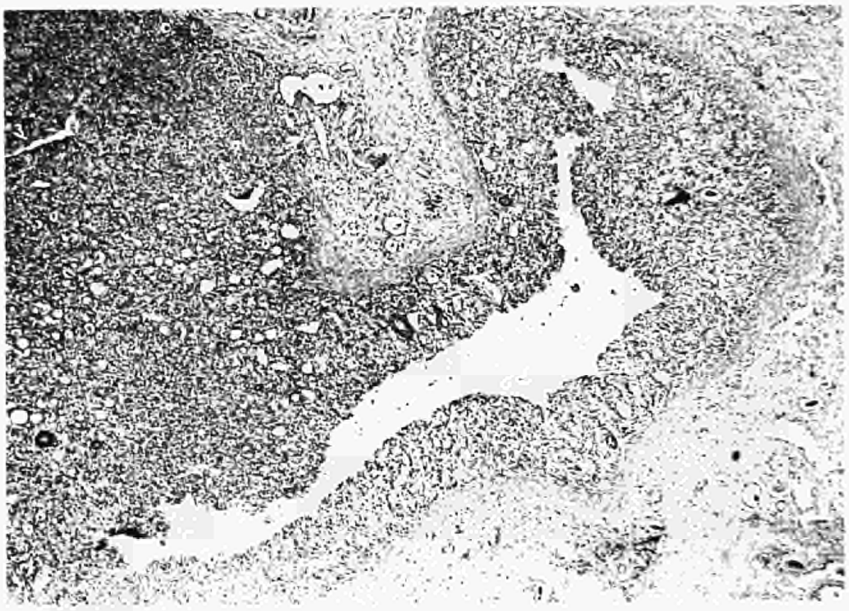
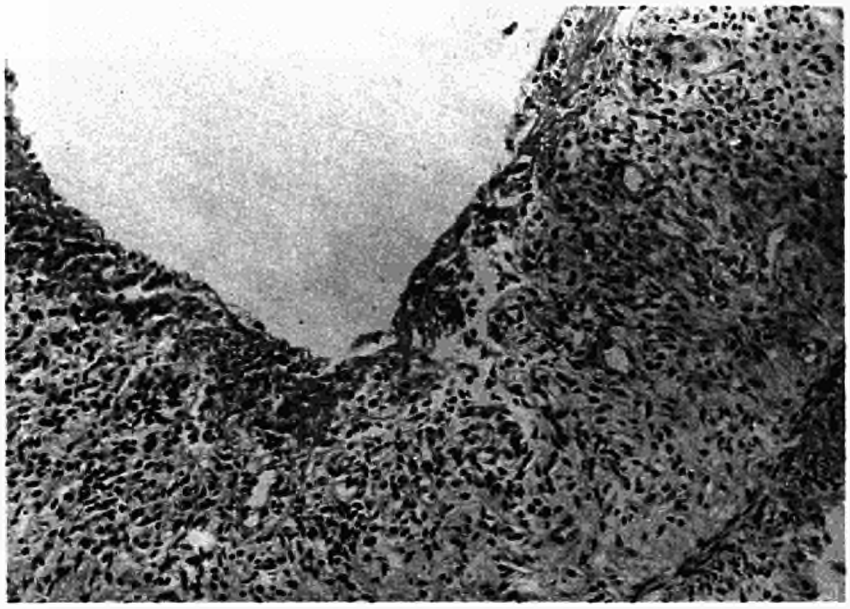


Plate 9 Complete loss of crypt and surface epithelium in ileum of leukemic child, 43 days after 950 r Co^{60} γ-irradiation and treatment with its mother's bone marrow, 12 and 15 days later (total of 14.5×10^9 cells). Surface of denuded mucosa is covered with fibrin and a few exudate cells.
Hematoxylin and eosin x 120
(courtesy of G. Mathé)

Plate 10 Cystic degeneration of mucosal glands of stomach in same monkey as in plate 8. Note desquamation of disintegrated cells and a completely atrophic gland in a centre of a focus of lymphoid cell infiltration to the right. Other glands have a hyperplastic appearance. Normal glands may be seen at left margin.
Hematoxylin and eosin x 120



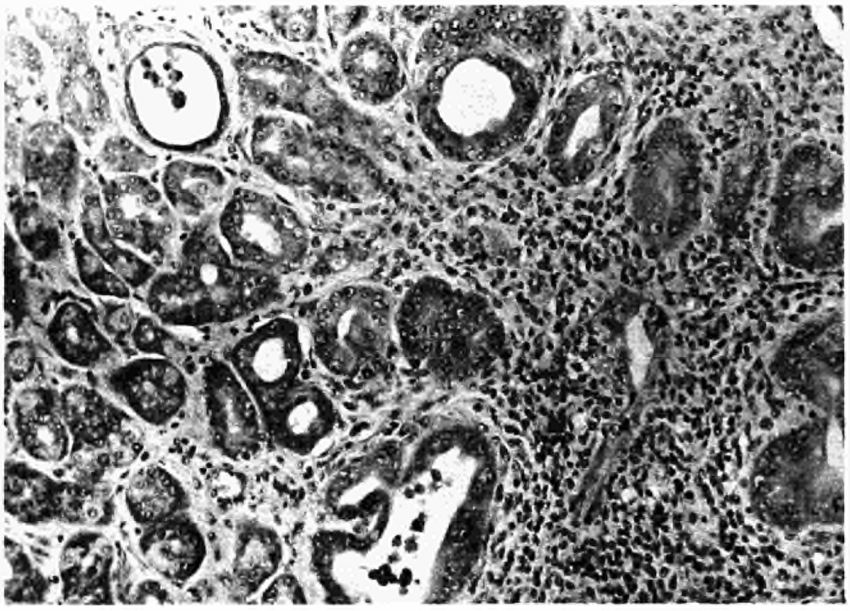
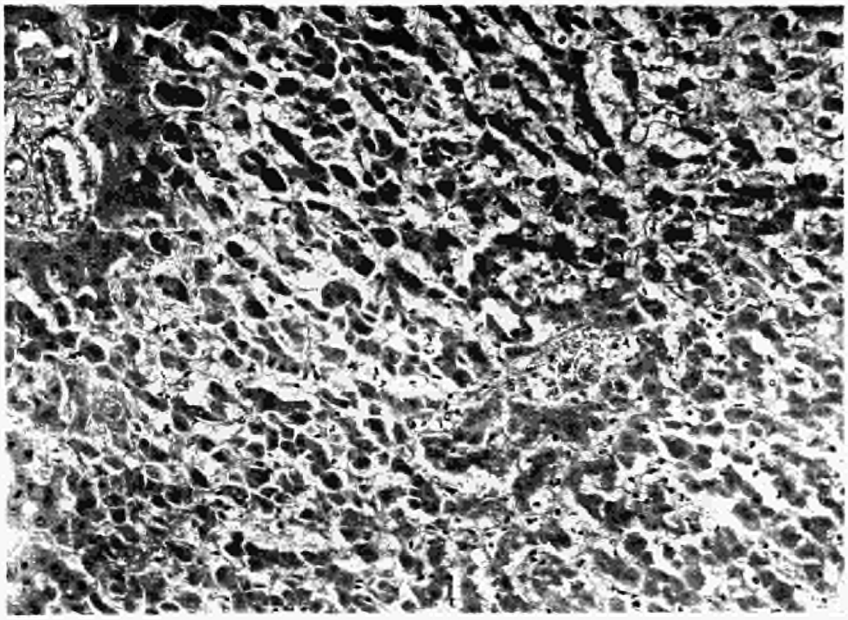


Plate 11 Dissociation and necrosis of hepatic parenchyma in monkey 23 days after irradiation (740 r) and treatment with homologous bone marrow (24×10^8 cells).
Hematoxylin and eosin x 120

Plate 12 Diffuse vacuolar degeneration of cells in esophageal epithelium of same monkey as in plate 7. A small vesicle containing degenerated cells is seen in the centre. Note lymphoid cell infiltration in lamina propria with diffuse extension into epithelial layer.
Hematoxylin and eosin x 120



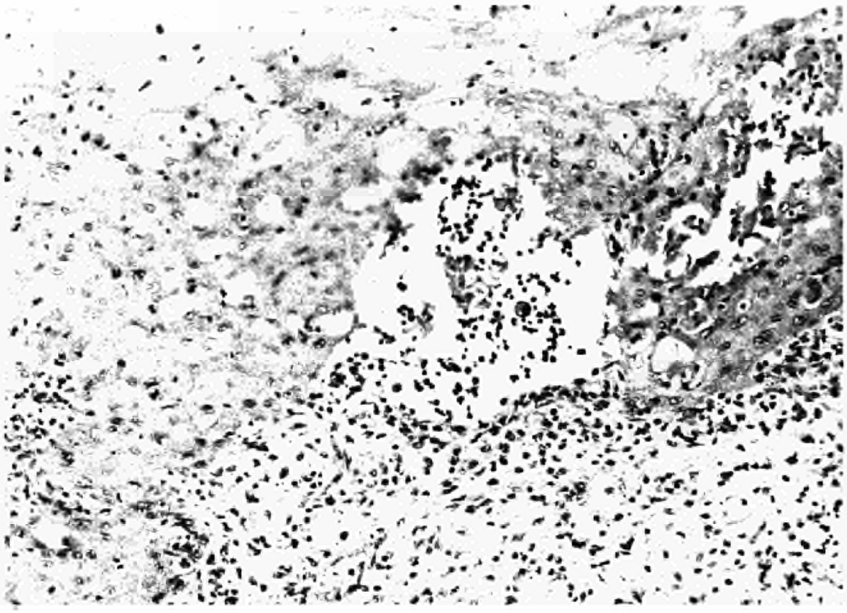


Plate 13 Karyorrhexxis and nuclear pyknosis in tubal epithelium of same monkey as in plate 8. A mitotic figure is seen in upper left corner.
Hematoxylin and eosin x 300

Plate 14 Karyorrhexxis in the epithelium of a medium-sized interlobular bile duct of same monkey as in plate 8. At the right margin a few necrotic liver cells with increased eosinophilia of cytoplasm are seen.
Hematoxylin and eosin x 190

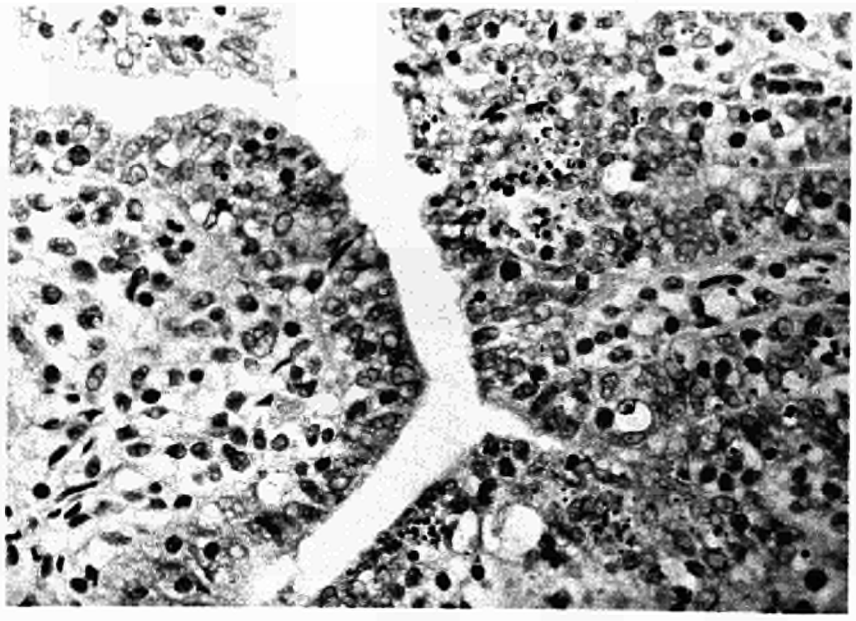
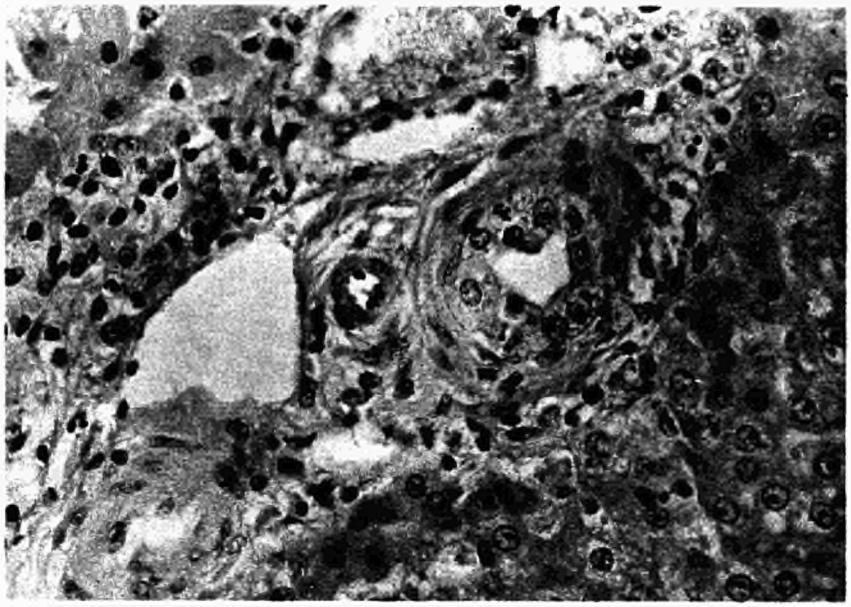


Plate 15 Periportal lymphoid cell infiltration in liver of same monkey as in plate 8. A few disintegrating epithelial cells are present in a small bile duct.
Hematoxylin and eosin x 300

Plate 16 Invasion of lymphoid cells into epithelium of hair follicles of same monkey as in plate 4.
Hematoxylin and eosin x 300



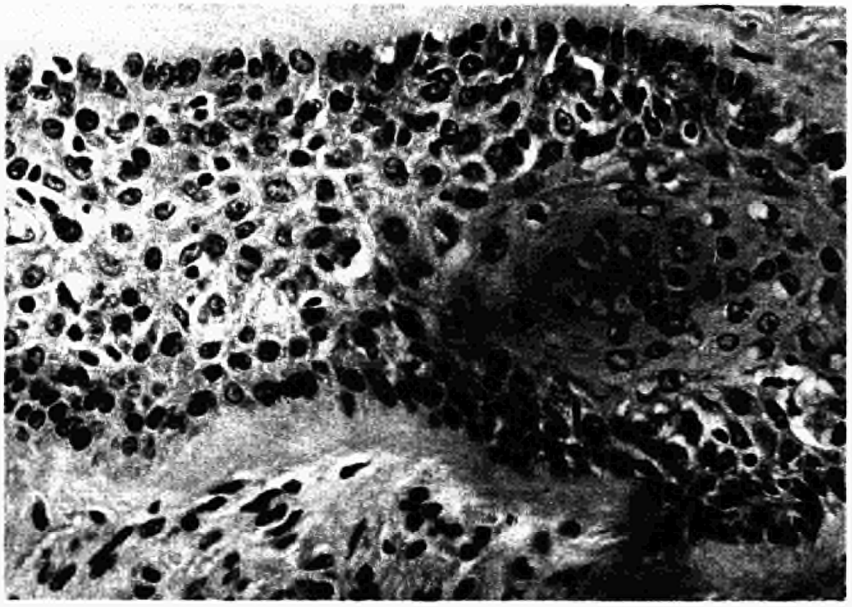
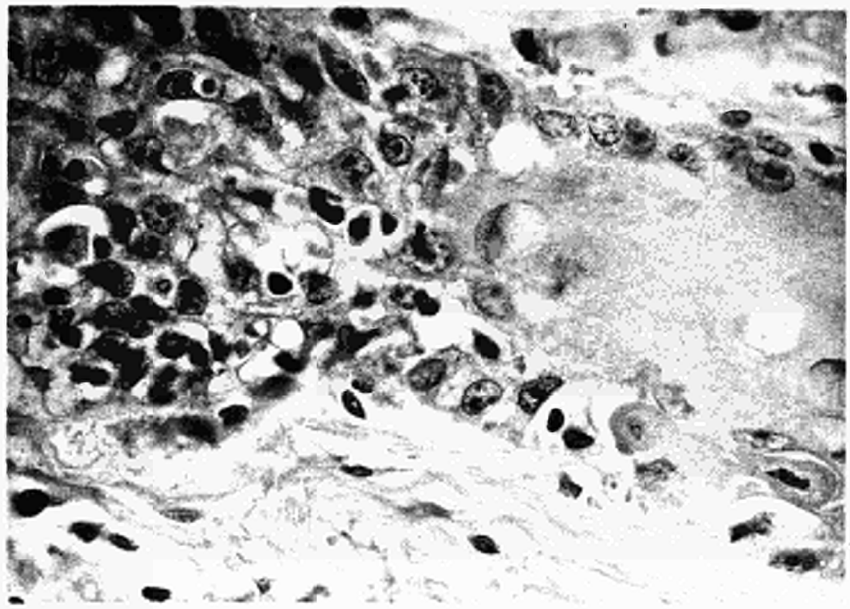


Plate 17 Pyknosis and vacuolar degeneration of epithelial cells adjacent to invaded lymphoid cells in hair follicle of same monkey as in plate 4. In lower right corner 2 dyskeratotic cells are seen. Hematoxylin and eosin. x 480

Plate 18 Radiation induced atrophy of lymph node of nontreated monkey 15 days after irradiation (810 r). A few collections of lymphocytes are still present in the depleted reticular stroma. Hematoxylin and eosin. x 120



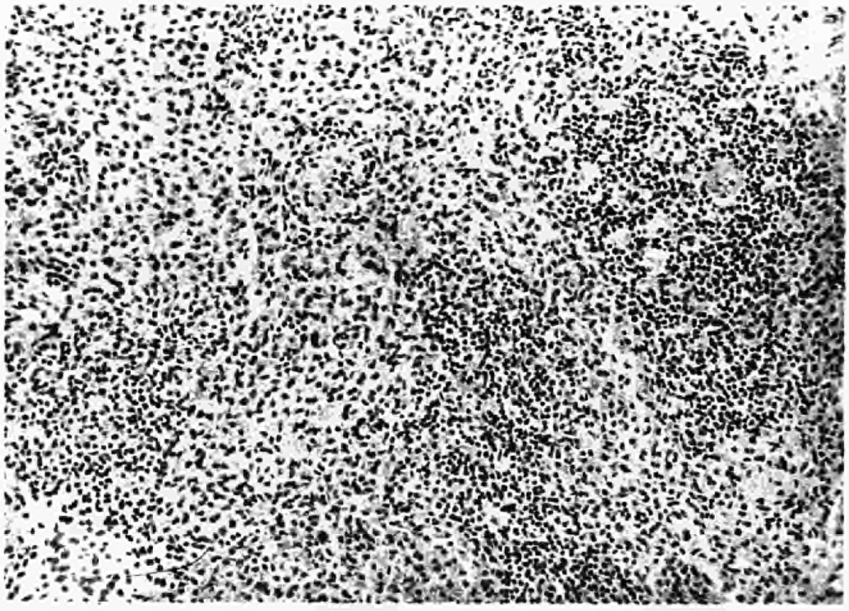
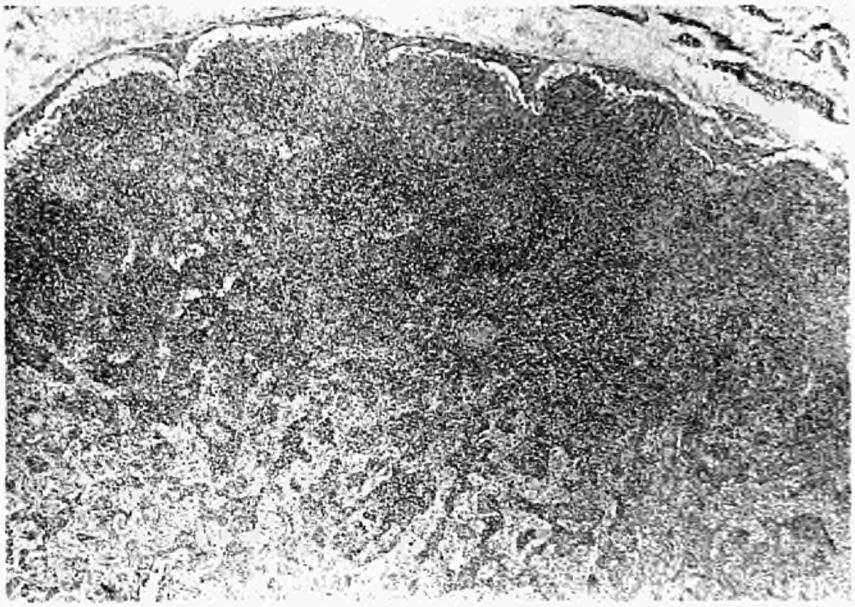


Plate 19 Regeneration in lymph node of same monkey as in plate 8. The cortex is crowded with lymphoid cells (see next plate).
Hematoxylin and eosin. x 30

Plate 20 Same lymph node as in plate 19. Extensive proliferation of reticular cells, stem cells and immature as well as mature lymphoid cells is apparent. Note several mitotic figures.
Hematoxylin and eosin. x 190



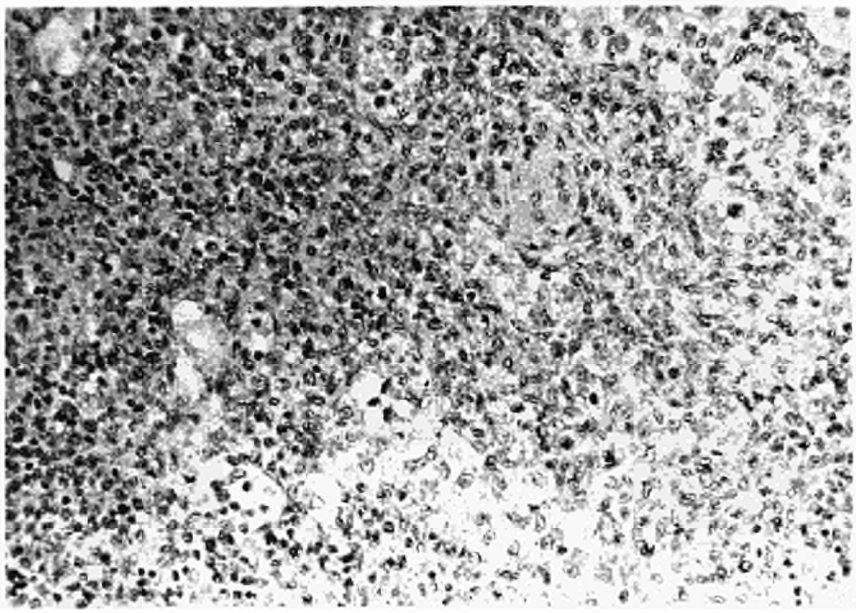


Plate 21 Wide-spread disintegration of lymphoid cells in lymphatic follicles and medullary tissue of lymph node of monkey 14 days after irradiation (650 r) and treatment with homologous bone marrow (22×10^8 cells).
Hematoxylin and eosin. x 190

Plate 22 Extreme atrophy of lymph node of monkey 47 days after irradiation and treatment with homologous bone marrow (22×10^8 cells). The cortex is more severely depleted of lymphoid cells than in control monkeys (compare with plate 18). The sinusoids are dilated.
Hematoxylin and eosin. x 30